Male infertility and intracytoplasmic sperm injection (ICSI)

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Micro-assisted fertilization in the form of intracytoplasmic sperm injection (ICSI) has truly revolutionised the treatment options for couples with impaired semen quality, and those with both obstructive and non-obstructive azoospermia. In general, the major issues which relate to the success of ICSI are those related to the success of conventional IVF, and the high multiple pregnancy rate remains a major cause for concern. There is growing evidence that the short-term health of ICSI offspring is relatively unremarkable, but our growing understanding of the genetic basis of much of male subfertility and of the impaired genomic integrity which characterises the oligozoospermic phenotype indicate a cautious approach to the longer term health of ICSI offspring.

Infertility remains a common problem causing significant psychological distress to those couples affected, who are increasingly seeking medical advice. The substantial contribution of reproductive dysfunction in the male partner to this infertility has been highlighted, and the development of micro-assisted fertilization techniques, principally intracytoplasmic sperm injection (ICSI), in the context of assisted conception has revolutionised the management of couples with so-called male factor infertility. This article focuses on the diagnosis and causes of male infertility and the development of ICSI to help such couples.

Infertility is commonly defined as the failure of conception after at least 12 months of unprotected intercourse; however, accurate assessment of the prevalence of infertility has always been difficult because of the scarcity of large-scale population based studies. Estimates suggest that some 14–17% of couples may be affected at some time in their reproductive lives, with recent European data suggesting that as many as one in four couples may experience difficulties in conceiving.

Contribution of the male partner to infertility

The accurate diagnosis of male infertility is fraught with problems and, therefore, establishing the extent of the male partner’s contribution to...
infertility is difficult. The majority of studies of the epidemiology and aetiology of male infertility have used the criteria of semen quality devised by the World Health Organization\textsuperscript{7-9}. However, a significant proportion of men with a normal semen analysis according to these criteria will be infertile because of defective sperm function\textsuperscript{10,11}, whilst a proportion of men with abnormal ‘WHO’ semen parameters will have normal sperm function\textsuperscript{12,13}. Hence, most studies use descriptive as opposed to functional diagnostic criteria, and it is becoming clear from recent studies on semen quality in relation to the achievement of spontaneous pregnancy that these descriptive criteria may be in urgent need of revision\textsuperscript{14}. Despite these shortcomings, numerous workers have concluded that the single most common factor contributing to infertility is abnormal semen quality\textsuperscript{5,6,15-19}.

**Diagnosis and classification of ‘male infertility’**

From the above, it can be seen that a major obstacle to the understanding and, therefore, treatment of male infertility is its accurate detection. It is important to emphasise the value of a proper clinical history and examination, as few positive diagnoses in clinical andrology can be made on the examination of semen samples alone\textsuperscript{20}. Whilst the WHO has devised a diagnostic classification of male infertility to introduce standardisation and facilitate multi-centre research, recent advances in understanding the genetic causes of male infertility argue for a review of the current classification\textsuperscript{21-25}. Diagnosis is traditionally based on criteria of semen quality promulgated by internationally recognised guidelines\textsuperscript{9-26}, which incorporate information on ejaculate volume, sperm concentration, graded sperm motility and morphological appearance. This complex assessment is subject to a range of shortcomings which might be expected to limit its diagnostic value. Many attributes of semen quality examined may be subjective in interpretation, although the recent introduction of quality control into routine diagnostic laboratory andrology practice should serve to limit inconsistencies in values between different laboratories\textsuperscript{27-29}. Additionally, marked inter ejaculate variation in semen quality within one individual is well recognised, and requires the assessment of multiple samples for diagnostic purposes\textsuperscript{8,30,31}. Lastly, it has become apparent that there is substantial geographical variation in human semen quality\textsuperscript{32}, so while the most recent WHO guidelines provide reference ranges for ‘normal’ values, these are not well evidence-based and highlight the importance of individual laboratories establishing normal values for their own local populations.

Given the difficulties which beset the meaningful diagnosis of ‘male infertility’ it is perhaps unsurprising that progress in understanding its
aetiology has been slow and that effective treatments are few. The clear exception to this is the application of techniques of micro-assisted fertilization, principally intracytoplasmic sperm injection (ICSI), in the context of assisted conception, which have revolutionised the management of couples with male factor problems.

**IVF and male infertility**

Early developments in *in vitro* fertilization (IVF) focused on couples with female factor infertility and particularly women suffering from bilateral tubal occlusion. Conventional IVF rapidly became established as an effective treatment option for couples with tubal disease and with unexplained infertility; however, it soon became apparent that it yielded generally poor pregnancy rates for couples with male factor infertility.

Tournaye et al., for example, compared *in vitro* fertilization and embryo transfer (IVF-ET) in a group of couples with male infertility and a similar group with tubal infertility. In cases of male infertility, more oocytes were recovered but fewer oocytes were fertilized, fewer embryo transfers were performed, the average number of embryos per transfer was lower and the total pregnancy rate per cycle was also lower at 12.8% versus 22.9%. They concluded that male infertility could be treated by IVF-ET, but that the results were disappointing when compared to a control group with normal spermatozoa. Although there was much discussion in the literature on the fine-tuning of the IVF procedure for couples with problems in the male partner, management options for couples with poor semen quality remained very limited until the breakthrough of effective micro-assisted fertilization in 1992.

**Micro-manipulation techniques**

Although the successful clinical application of techniques of micro-assisted fertilization took place some 14 years after the first successful human *in vitro* fertilization, animal experimentation with micro-assisted fertilization had in fact pre-dated human IVF. In early human studies, a range of approaches was initially explored.

*Partial zona dissection*

Partial zona dissection (PZD) was the first micromanipulation technique studied in animal models with clinical intent, and early reports in human practice of clinical pregnancies were encouraging, suggesting
that monospermic fertilization and cleavage rates could be doubled by these approaches. However, concerns existed over the risk of polyspermy, along with doubts about appropriate case selection.

Sub zonal insemination

Sub zonal insemination (SUZI) involves the injection of spermatozoa into the perivitelline space and, again, initial reports of its use were encouraging, although other groups found the technique to be less successful.

Intracytoplasmic sperm injection

The developments in human micro-assisted fertilization culminated in ICSI, with the first human pregnancies resulting from this technique being described by the Brussels group in 1992. This approach involves injection of a single spermatozoon directly into the cytoplasm of the oocyte through the intact zona pellucida, and it very soon became apparent that this technique produced superior results to PZD or SUZI, with pregnancy rates of 22% per started cycle being reported. Indeed, such has been the success of ICSI that commentators have been moved to suggest that it might be considered the treatment of choice for all cases where in vitro conception is indicated.

Verheyen et al. reported on a controlled comparison of conventional IVF and ICSI in patients with asthenozoospermia (which they defined as <5% rapidly progressive spermatozoa) and noted a very much reduced fertilization rate and substantial risk of failure of fertilization associated with the use of conventional IVF. The same group reported on a study comparing IVF with ICSI in sibling oocytes from couples with tubal infertility and normal semen quality. They observed that the use of ICSI in this group was not detrimental to embryo quality or implantation potential. The results of a large multi-centre randomized controlled trial comparing IVF with ICSI in normozoospermic couples are eagerly awaited. Most recently, a meta-analysis has concluded that, for couples with normal semen, there is no evidence of any benefit either in fertilization rates per retrieved oocyte, or in pregnancy rates, between ICSI and conventional IVF. In contrast, for couples with borderline semen, ICSI results in higher fertilization rates than IVF, and couples with very poor semen will have better fertilization outcomes with ICSI than with SUZI or additional IVF.

ICSI with epididymal spermatozoa

Initially, clinical ICSI was used in the treatment of couples in whom the male partner had substantially abnormal semen quality, but it was not long...
before the technology was applied to the significant numbers of men who present with no sperm in their ejaculates. Amongst men with obstructive azoospermia, attention focused on spermatozoa derived from the epididymis. The first pregnancies achieved with epididymal sperm were described using conventional IVF\textsuperscript{57,58}. However, fertilisation rates were low, so whilst it was established that sperm from the epididymis had a degree of functional competence, this was limited\textsuperscript{59,60}, and animal experimentation suggested that micro-assisted fertilization was likely to be beneficial\textsuperscript{42,61}. Initially, the use of SUZI was described\textsuperscript{62,63}, but this was rapidly replaced in clinical practice by ICSI\textsuperscript{64,65}, which achieved very satisfactory success rates.

As a consequence of the successful use of epididymal spermatozoa in ICSI, techniques have been described to facilitate the surgical retrieval of spermatozoa\textsuperscript{66,67}. The major approaches include microsurgical epididymal sperm aspiration (MESA), and percutaneous epididymal sperm aspiration (PESA). MESA involves a formal scrotal exploration, is commonly performed under general anaesthetic, and hence is a significant surgical intervention\textsuperscript{68}. A major advantage of this technique is that it permits full scrotal examination and, therefore, has diagnostic power. The number of spermatozoa retrieved is high, which facilitates cryopreservation\textsuperscript{69} and, if indicated, it can be combined with definitive reconstructive surgery, such as vaso-vasostomy or epididymo-vasostomy\textsuperscript{70}. PESA is a widely used technique which is less invasive, can be performed under local anaesthesia\textsuperscript{71-73}, and can be performed repeatedly\textsuperscript{74}. PESA provides less diagnostic information, and the yield of spermatozoa may be lower, with one recent review suggesting that at least 20\% of attempts at PESA are unsuccessful and require resort to MESA\textsuperscript{66}. It has also been argued that, in patients with presumed obstructive azoospermia, direct testicular fine needle aspiration (TFNA) may be the procedure of choice\textsuperscript{67}.

**ICSI with testicular spermatozoa**

In contrast to the position in men with obstructive azoospermia, amongst men with non-obstructive azoospermia attention naturally focuses on the testis as a site for sperm recovery. With the availability of ICSI, it has become clear that non-obstructive azoospermia is a very heterogeneous condition, and that testicular histology is similarly heterogeneous, with foci of apparently normal spermatogenesis adjacent to seminiferous tubules devoid of germ cells\textsuperscript{75}. These observations led to attempts at the surgical recovery of sperm from men with non-obstructive azoospermia, and the successful achievement of pregnancies\textsuperscript{76}. Since these exciting initial observations, surgical sperm recovery from men with non-obstructive azoospermia has become a routine part of clinical infertility practice\textsuperscript{77-81}.
and, as with the epididymis, cryopreservation of testis derived spermatozoa has also become routine. A recent review concluded that surgical sperm recovery would be successful in some 48% of men with non-obstructive azoospermia.

Various approaches to the surgical recovery of sperm from the testis have been described, both in the context of obstructive and non-obstructive azoospermia. Testicular sperm aspiration (TESA) is a simple percutaneous aspiration technique, performed under local anaesthetic, and which has a high success rate of sperm retrieval in those men with normal spermatogenesis. The advantages of TESA are its simplicity, quickness and non-invasiveness. Testicular sperm extraction (TESE) on the other hand involves an open excisional biopsy, equivalent to a diagnostic biopsy. Sperm are extracted using either mincing techniques or enzymatic digestion to disrupt the tissue and allow seminiferous tubules to release spermatozoa. TESE also allows cryopreservation of tissue. Girardi and Schlegel and Tournaye in recent reviews of the subject have argued that in men with non-obstructive azoospermia, surgical access to the testis is required, since pockets of spermatogenesis are isolated, and larger or multiple biopsies are more often needed. This view has been supported by recent comparative studies in which TESE was required in almost 80% of men with non-obstructive azoospermia.

Undoubtedly, one of the major problems confronting the process of surgical sperm recovery from men with non-obstructive azoospermia is the fact that there are currently no good predictors of which patients will have sperm recovered successfully, and which will not. Against this background, a number of groups have argued that surgical recovery of sperm from the testis coupled with cryopreservation should precede ovarian stimulation in the female partner.

For those men in whom mature spermatozoa cannot be recovered, there is currently interest in the possibility of using less mature cells, commonly elongating or round spermatids, to achieve fertilization. Work in animal models has suggested that this may be a viable approach, and there are a number of clinical case reports in the literature. At the present time, however, uncertainties over the safety and efficacy of this approach should confine its use to properly designed clinical trials.

Success rates of ICSI

ICSI has become well established as an effective form of treatment for couples with male factor infertility. In the last year for which data are available (1997/98) the UK’s Human Fertilization and Embryology Authority reported 9295 cycles of ICSI, alongside 24,889 cycles of IVF – in other words, some 27% of assisted conception treatment in the UK.
is for severe male factor infertility. In the same year, the reported live birth rate for ICSI was 20.7% per cycle compared to 14.9% for IVF. In keeping with the major issues in assisted conception in general, the multiple birth rate was high at over 25%, and the age of the female partner was a major determinant of success, with a woman in her early thirties achieving a 20–25% per cycle success rate, dropping to 5% or less for a woman over the age of 40 years.

Tarlatzis recently reviewed the world-wide experience of ICSI. He noted that over the 3 year period (1993–1995), the number of centres undertaking ICSI in Europe increased from 35 to 101, and the total number of ICSI cycles per year rose from 3157 to 23,932. The incidence of oocytes damaged by the procedure was low (< 10%), and the fertilization rates obtained with ejaculated, epididymal, and testicular spermatozoa for 1995 were 64%, 62%, and 52%, respectively. Ultimately, between 86–90% of couples achieved embryo transfer, and the viable pregnancy rate was 21% for ejaculated, 22% for epididymal, and 19% for testicular sperm, with an incidence of multiple gestations of 29%, 30%, and 38%, respectively. It was noteworthy that no difference was found in ICSI results concerning the aetiology of azoospermia; for example, obstructive (congenital or acquired) or non-obstructive. The Brussels group have recently reviewed their own data on ICSI outcomes in a group of Belgian couples, aged less than 37 years, who had their first ICSI cycle between 1992/93. The per cycle delivery rate was high at 31%, but the real cumulative pregnancy rate was 60% after 6 cycles of treatment. Again, female age had a powerful effect on success rates.

**Outcomes of ICSI**

Given that ICSI is effective, is it also safe? The rapid development of micro-assisted conception techniques and the wide-spread use of in ICSI in alleviating male infertility have raised concerns about the health of the offspring. ICSI, by directly injecting individual spermatozoa into a mature oocyte bypasses the natural physiological processes of normal sperm selection, raising concerns over the potential risk of congenital malformations and genetic defects in children born after ICSI. It has also stimulated debate regarding associations between obstetric and perinatal outcome and type of conception. Recent work showing that poor quality semen tends to contain gametes with compromised DNA integrity, but that this does not compromise its fertilizing ability at ICSI further highlights the importance of interest in the area of ICSI outcome.

Without doubt, the most thorough and detailed follow-up studies of ICSI offspring have been those orchestrated by the Brussels group who have undertaken a prospective follow-up study of 1987 children born after ICSI,
aiming to compile data on karyotypes, congenital malformations, growth parameters and developmental milestones. They were able to collect data on 1699, 91 and 118 children born after ICSI with ejaculated, epididymal and testicular spermatozoa, respectively, as well as on 79 children born from cryopreserved ICSI embryos. Of 1082 karyotypes determined by prenatal diagnosis, 1.66% (18) were abnormal de novo (nine each of autosomal and sex chromosomal aberrations), and 0.92% (10) were inherited structural aberrations. Of these, nine (eight balanced structural aberrations and one unbalanced trisomy 21) were transmitted from the father. Forty-six major malformations (2.3%) were observed at birth. Seven malformations, observed by prenatal ultrasound, were terminated. Twenty-one (1.1%) stillbirths, including four with major malformations, occurred later than 20 weeks of pregnancy.

In Denmark, a national cohort study of 730 infants born after ICSI99 included all clinical pregnancies obtained after ICSI registered in Denmark between January 1994 and July 1997 at five public and eight private fertility clinics. Only 183 women (28.5%) underwent prenatal diagnosis, resulting in 209 karyotypes with seven (3.3%) chromosome aberrations. Six major chromosomal abnormalities (2.9%) and one inherited structural chromosome aberration (0.5%) were found, but no sex chromosome aberrations. The frequency of multiple birth, Caesarean section rate, gestational age, preterm birth, and birth weight were comparable with previous studies. The perinatal mortality rate was 13.7 per 1000 children born with a gestational age of 24 weeks or more. In 2.2% of the live-born infants, and in 2.7% of all infants, the parents reported major birth defects. Minor birth defects were found in nine live-born infants (1.2%).

Most recently, Wennerholm et al100 studied the incidence of congenital malformations in a complete cohort of 1139 infants, 736 singletons, 200 sets of twins and one set of triplets born after ICSI. The number of infants with an identified anomaly was 87 (7.6%), 40 of which were minor. The incidence of malformations in children born after ICSI was compared with all births in Sweden using data from the Swedish Medical Birth Registry and the Registry of Congenital Malformations. For ICSI children, the odds ratio (OR) for having any major or minor malformation was 1.75 (95% confidence interval (CI) 1.19–2.58) after stratification for delivery hospital, year of birth and maternal age. If stratification for singletons/twins was also done, the OR was reduced to 1.19 (95% CI, 0.79–1.81). The increased rate of congenital malformations is thus mainly a result of a high rate of multiple births. Of interest, the only specific malformation which was found to occur in excess in children born after ICSI was hypospadias (relative risk 3.0, exact 95% CI, 1.09–6.50). The same group examined the obstetric outcome of pregnancies after ICSI101. Deliveries occurred in 75.9% and early spontaneous abortion, late spontaneous abortion and ectopic pregnancy in 21.4%, 1.0% and 1.2% of pregnancies, respectively.
Multiple birth occurred in 21.3% (almost all twins) of deliveries, and preterm birth occurred in 15.7% of all deliveries. Preterm birth was not related to sperm origin or quality but was related to multiple birth. The prematurity rate was 8.4%, 42.3% and 100% for singletons, twins and triplets, respectively. The perinatal mortality rate was 11.7 per 1000 born infants; 7.3% of infants had a malformation, 40 of which were minor. They concluded that the obstetric outcome of ICSI pregnancies was similar to that of conventional IVF and was not influenced by sperm origin or quality. The high incidence of multiple births remains the major concern. Other groups have reached similar conclusions.

In other words, as the database of ICSI offspring grows larger, the available evidence on the short-term health of these offspring is generally reassuring. It is important, however, to appreciate the important role that a genetic aetiology plays in the origins of much male subfertility, and the ability of ICSI to promote the transgenerational transmission of genetic defects causing gametogenic failure. It seems clear that male, but not female, fertility problems show a distinct pattern of familial aggregation, and that couples with male infertility have fewer siblings than fertile controls. The significantly increased risk of chromosomal abnormalities in men with impaired semen quality is easily managed by the appropriate investigation and counselling which are required prior to treatment.

Some groups have even advocated chromosomal studies on both partners. It is less easy to be certain how to respond to the available evidence on microdeletions of the Y chromosome in men with severely impaired semen quality. The strength of the association between Y chromosome deletions and severely impaired semen quality is impressive, and it is increasingly suggested that these lesions may result in progression from oligozoospermia to azoospermia over time. In a recent study, Pryor et al. found deletions in 7% of infertile men, and in only 2% of normal men, but observed no clear relationships between the size and location of the deletions and the severity of the spermatogenic failure. Moreover, it is clear that these genetic deletions, if present, can be transmitted to offspring via ICSI.

On the basis of this evidence, some authorities now advocate screening of men for Y chromosome microdeletions prior to ICSI, and advocate testing of offspring and reproductive monitoring for those found to have inherited deletions.

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